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Spontaneous Mitotic Recombination in Yeast: The Hyper-Recombinational *rem1* Mutations Are Alleles of the *RAD3* Gene

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ABSTRACT

The *RAD3* gene of *Saccharomyces cerevisiae* is required for UV excision-repair and is essential for cell viability. We have identified the *rem1* mutations (enhanced spontaneous mitotic *rec*ombination and *m*utation) of *Saccharomyces cerevisiae* as alleles of *RAD3* by genetic mapping, complementation with the cloned wild-type gene, and DNA hybridization. The high levels of spontaneous mitotic gene conversion, crossing over, and mutation conferred upon cells by the *rem1* mutations are distinct from the effects of all other alleles of *RAD3*. We present preliminary data on the localization of the *rem1* mutations within the *RAD3* gene. The interaction of the *rem1* mutant alleles with a number of radiation-sensitive mutations is also different than the interactions reported for previously described (UV-sensitive) alleles of *RAD3*. Double mutants of *rem1* and a defect in the recombination-repair pathway are inviable, while double mutants containing UV-sensitive alleles of *RAD3* are viable. The data presented here demonstrate that: (1) *rem1* strains containing additional mutations in other excision-repair genes do not exhibit elevated gene conversion; (2) triple mutants containing *rem1* and mutations in both excision-repair and recombination-repair are viable; (3) such triple mutants containing *rad52* have reduced levels of gene conversion but wild-type frequencies of crossing over. We have interpreted these observations in a model to explain the effects of *rem1*. Consistent with the predictions of the model, we find that the size of DNA from *rem1* strains, as measured by neutral sucrose gradients, is smaller than wild type.

In baker's yeast, *Saccharomyces cerevisiae*, a number of genes (*RAD* genes) involved in DNA repair have been identified by mutations that confer ultraviolet (UV) or X-ray sensitivity (for reviews see HAYNES and KUNZ 1981; GAME 1983). Three major dark repair pathways, or epistasis groups, have been identified. The pathways (named for a major participating gene) are: (1) excision-repair of UV-induced thymine dimers and other bulky lesions (*RAD3*), (2) double-strand break (dsb) or recombination-repair (*RAD52*), and (3) error-prone or mutational repair (*RAD6*). The *RAD3* gene has been of particular interest since it was discovered that disruption or deletion of the coding region is lethal in haploid cells (HIGGINS *et al.* 1983; NAUMOVSKI and FRIEDBERG 1983). In this paper we report a hitherto unknown class of mutations in *RAD3* that do not cause extreme UV sensitivity but rather change the properties of DNA metabolism as evidenced by increased levels of mitotic *rec*ombination and spontaneous *m*utation (hence their original designation, *rem1* mutations). (Note: "Mitotic recombination" as discussed in this paper refers to recombination between homologs.)

The *rem1* mutations confer a semidominant, mitosis-specific, hyper-rec/hyper-mutable phenotype (GOLIN and ESPOSITO 1977, 1981; MALONE and HOEKSTRA 1984). The first allele, *rem1-1*, was isolated as a mutator and subsequently shown to increase spontaneous mitotic recombination (GOLIN and ESPOSITO 1977, 1981). We independently isolated a second allele, *rem1-2*, as a hyper-rec mutation (MALONE and HOEKSTRA 1984), and have shown it to confer a mutator phenotype (HOEKSTRA and MALONE 1987). Unlike certain *rad* mutations that can display some of the *rem1* phenotypes, strains containing *rem1* are essentially as resistant as wild-type cells to treatments such as UV and methyl methanesulfonate (MMS) (HOEKSTRA and MALONE 1987). That is, *rem1* mutations do not appear to confer a significant defect in repair.

The effects of the *rem1* alleles on recombination have been extensively studied (GOLIN and ESPOSITO 1977, 1981; MALONE, GOLIN and ESPOSITO 1980; MALONE and HOEKSTRA 1984). The distribution of recombination events along a chromosome in *rem1* strains is intermediate to wild-type mitotic and meiotic distributions (MALONE, GOLIN and ESPOSITO 1980). We demonstrated by multiple mutant analysis that inappropriate expression of the meiotic recombination system seem unlikely to be responsible for the *rem1* phenotype (MALONE and HOEKSTRA 1984). We also found that the double mutants *rem1 rad50* and

TABLE 1

Phenotypes of mutations used in combination with *rem1*

Mutation	Radiation sensitivity	Repair group[a]	Recombination: Spontaneous[c] mitotic	Recombination: Meiotic[d]	Spontaneous[b] mutation	Comments
rem1	±UV[e]	NA[f] (ER)	+ + +	+	+ + +	Semidominant hyper-rec, mutator
rad1	UV	*RAD3* ER	+	+	±	Deficient in dimer removal
rad4	UV	*RAD3* ER	+	+	±	Deficient in dimer removal
rad50	X/γ	*RAD52* DSBR	+ + +	0	+ +	Sporulation defective; meiotic Rec⁻
rad52	X/γ	*RAD52* DSBR	0	0	+ +	Sporulation defective; general Rec⁻

Information summarized from reviews by HAYNES and KUNZ (1981) and GAME (1983).
[a] ER is excision repair; DSBR is double-strand break repair.
[b] ± = slightly lower than wild type; + + and + + + = varying levels of increased mutation.
[c] + = wild type; 0 = decreased levels; + + + = increased recombination.
[d] + = proficient; 0 = absent or reduced.
[e] *rem1* is slightly UV sensitive at high fluence levels.
[f] Not applicable.

rem1 rad52 are inviable [*RAD50*, like *RAD52*, is required for recombination-repair (GAME *et al.* 1980; MALONE and ESPOSITO 1980; PRAKASH *et al.* 1980; HAYNES and KUNZ 1981; GAME 1983)]. This observation led to the proposal that lesions occur in *rem1* strains that require recombination-repair for resolution. In this paper, we provide evidence that these lesions may be double strand breaks.

MATERIALS AND METHODS

Strains and culture conditions: The yeast strains used in this study are closely related isolates containing the various recombination and repair mutations described throughout the text and in Table 1. All strains have been backcrossed at least three times to the strains K210-4A, K210-6D, K264-5B, or K264-10D (MALONE and HOEKSTRA 1984). Haploids contain (some or all of) either of two sets of mutations which, when intercrossed, generate up to seven different heteroallelic and two heterozygous drug-resistance markers for the measurement of recombination. Haploid genotypes, for either mating type, were: (1) *ho lys2-1 tyr1-1 his7-2 can1^R ura3-13 ade5 met13-d trp5-2 leu1-12 ade2-1*; or (2) *ho lys2-2 tyr1-2 his7-1 ura3-1 met13-c cyh2^R trp5-c leu1-c ade2-1*. Strains not of these configurations are noted in the text. The *rad1-2*- and *rad3-2*-containing strains originated from L. PRAKASH (University of Rochester). The *rad4* mutation was obtained from the Yeast Genetic Stock Center (Berkeley, California).

Yeast media formulations and standard techniques for sporulation, dissection, testing of auxotrophic requirements, and segregation analysis have been described, as have procedures for determining recombination levels (MALONE and HOEKSTRA 1984).

The *Escherichia coli* strains used throughout the course of this work were HB101, MC1066, or RK1400 (obtained from R. KOLODNER) (SYMINGTON, FOGARTY and KOLODNER 1983). Media for growth of *E. coli* are described in MANIATIS, FRITSCH and SAMBROOK (1982).

Isolation of *RAD3*: Spheroplasts of the *ura3-52 rad3-2* strain LP2649-1A (HIGGINS *et al.* 1983) were transformed to uracil independence using a wild type yeast DNA pool in plasmid YEp24 (CARLSON and BOTSTEIN 1982; kindly provided by S. C. FALCO, E. I. du Pont de Nemours and Co.). The agar overlay containing the transformants was lifted off the regeneration plates and macerated in a small volume of 0.2 M Na_2HPO_4 buffer (pH 7.5). The mixture was diluted and transformants plated for single colonies on uracil omission medium. Of transformed colonies arising, 22,500 were picked to grid patterns on uracil omission medium, grown overnight at 30°, replicated to uracil omission medium and the replicates exposed to a UV light source (2 × 15 Watt G.E. model G15T8 Germicidal Lamps, fluence exposure of 100 J/m^2). The exposed plates were immediately wrapped in foil to avoid photoreactivation and grown for 2 days. After retesting resistant patches, five consistently demonstrated approximately wild-type levels of UV resistance. Included as controls on each plate were *RAD3* and *rad3-2* strains containing the vector, YEp24. All five UV-resistant clones demonstrated cosegregation of the plasmid with UV resistance.

The plasmids were rescued in *E. coli* from total yeast DNA preparations. Restriction analysis demonstrated that all five had the same insert. One of these, pMFH100, was chosen for subsequent analysis.

DNA manipulation: Restriction digestions followed the recommendation of manufacturers. Enzymes were purchased from Bethesda Research Laboratories (Gaithersberg, Maryland) and New England Biolabs (Beverly, Massachusetts). Procedures for transformation, DNA isolation, plasmid purification, and DNA blot hybridizations have

been described (MANIATIS, FRITSCH and SAMBROOK 1982; MALONE and HYMAN 1983; HOEKSTRA and MALONE 1985).

Rescue of *rem1* alleles by transformation with gapped plasmids: Haploid strains bearing *rem1-1* or *rem1-2* were transformed with derivatives of pMFH102 lacking the *Hpa*I internal fragments (this gap removes the entire *RAD3* coding region), the *Cla*I internal fragment, the *Sma*I to *Cla*I fragment, or the *Cla*I to *Bal*I fragment (see Figure 1). Transformants containing a plasmid the size of the starting full-length pMFH102 were picked and total yeast DNA prepared. This DNA was used to transform *E. coli*. Plasmid DNA was prepared from the transformants and shown to have the restriction map expected of a faithful gap-rescue of the *RAD3* region. This plasmid DNA was transformed into a diploid yeast strain wild type for all repair genes which contained diagnostic markers to monitor gene conversion and crossing over (see MATERIALS AND METHODS).

Determination of mitotic recombination frequencies: All measurements of mitotic recombination were done with freshly mated diploids using the procedure described in MALONE and HOEKSTRA (1984).

Determination of the contribution of chromosome loss to drug-resistance frequencies: The frequency of drug-resistant colonies arising from a population of sensitive diploid cells heterozygous for a recessive drug-resistance marker is used as a measure of the frequency of crossing over between the marker and its centromere. Since chromosome loss may also contribute to the resistant population, we employed strains specially marked to determine the extent of this contribution. Diploid strains were heterozygous for $can1^R$ (canavanine-resistance) on the left arm of chromosome *V*, linked in coupling to *his1* on the right arm. Canavanine-resistant colonies that result from crossing over will remain histidine independent, while those resulting from loss of the homolog bearing the sensitivity allele will become auxotrophic for histidine. Similarly, to assess loss of chromosome *VII*, our strains were heterozygous for $cyh2^R$ and *ade6* and homozygous for *ade2-1*. Among Cyh^R colonies, mitotic recombinants are red whereas white colonies result from chromosome loss (see ROMAN 1956).

At least 200 single colonies of freshly mated diploids were picked onto YPD master plates in patches. These plates were replicated onto canavanine- or cycloheximide-containing media in a manner to produce well-separated papillae. A single papilla was picked from each patch and tested for expression of the recessive marker on the other side of the centromere as described above.

Sucrose gradient analysis: The wild-type and *rem1* strains used were the products of five rounds of backcrosses and were therefore 97% isogenic. The procedure used for sucrose gradient analysis of yeast chromosomal DNA was that of RESNICK *et al.* (1981, 1984) and RESNICK, BOYCE and COX (1981). Briefly, cells were grown overnight in complete synthetic medium containing 12.5 μg/ml adenine and 10 μCi of [^{3}H]adenine or [^{14}C]adenine (Research Products International, Chicago, Illinois). Where indicated, the label was chased for one generation in synthetic medium containing 50 μg/ml adenine. Gentle cell lysis was accomplished by incubating cells in 0.1 M Tris-sulfate (pH 9.3), 0.01 M EDTA, 0.3 M 2-mercaptoethanol for 10 min at 37°, washing and resuspending cells in 50 mM K_2HPO_4 (pH 6.5), 10 mM EDTA (at 10^8 cells/ml) and adding 2×10^7 cells to 20 μl of 12.5% Na-Sarkosyl, 20 μl of 2 mg/ml RNAse A, 20 μl of 2 mg/ml Zymolyase 60,000. The mixture was incubated at 37° for 10 min in a 1000 μl pipetor tip which had been shortened to enlarge the bore and sealed with parafilm. Ten microliters of 5 mg/ml Proteinase K were added to the mixture and held for 30 min. Just prior to loading, 50 μl of a solution containing 20 mg/ml Na-Sarkosyl, 30 mg/ml Na-deoxycholate, 50 mg/ml Na-lauryl sulfate were added to complete lysis. Pre-formed 5–20% linear gradients were gently loaded by placing the pipetor tip on an automatic pipet gun and slowly dialing the lysed cells on the gradient. Centrifugation was in an SW50.1 rotor at 9,000 rpm. for 16 hr.

Gradients were fractionated from the bottom and each fraction made to 0.3 M NaOH, incubated at 37° for 60 min, neutralized with HCl and an equal volume of ice cold 10% TCA added. The precipitate was collected on Whatman glass fiber filters, dried, and counted using a toluene-based scintillation cocktail. Measurements of radioactivity were performed using a Unilux II (Nuclear Chicago) or a LS-5801 (Beckman) liquid scintillation counter.

RESULTS

Genetic data indicating *rem1* is an allele of *RAD3*: In the course of genetic crosses to construct *rem1 rad4* double mutant strains, we noticed that the parental class of tetrads greatly exceeded nonparental or tetratype tetrads. Because *RAD3* is linked to *RAD4* at a distance of 16.4 cM (MORTIMER and SCHILD 1980), we examined the linkage of *rem1* to both *RAD3* and *RAD4*. The failure to observe any recombinants between *rem1* and *rad3* suggested that the *rem1* mutations might be alleles of the *RAD3* gene (Table 2).

Analysis of a cloned *RAD3* gene: To determine if *REM1* and *RAD3* were the same gene, we cloned *RAD3* to test for complementation of the *rem1* phenotype. Spheroplasts of the genotype *ura3-52 rad3-2* were transformed to uracil independence using a wild-type yeast DNA pool as described in MATERIALS AND METHODS. Transformants were tested for UV resistance and clones demonstrating wild-type UV resistance were chosen for further use. A plasmid, pMFH100, was isolated in *E. coli* that, upon retransformation of yeast, complemented the *rad3-2* mutation for UV sensitivity. The plasmid did not complement other UV-sensitive mutations, such as *rad1-2* (data not shown). The restriction map of the pMFH100 insert is given in Figure 1; it is identical to the map of *RAD3* published by NAUMOVSKI and FRIEDBERG (1983) and HIGGINS *et al.* (1983). The plasmids pMFH100, pMFH102 (a subclone containing the *Kpn*I-*Sal*I fragment), and pNF3001 [a *RAD3* plasmid provided by L. NAUMOVSKI and E. FRIEDBERG (NAUMOVSKI *et al.* 1985)] were tested for their ability to complement the *rad3* and *rem1* mutant phenotypes. All three plasmids were able to eliminate *rad3* UV sensitivity and reduce *rem1* hyper-recombination (Figure 1 and Table 3). We conclude that *rem1-1* and *rem1-2* are alleles of the essential yeast excision-repair function *RAD3*. The effect of *RAD3* gene dosage is also seen in Table 3: the centromere-containing plasmid pNF3001 (which would be present in two copies per diploid cell, on the average) consistently showed less of a reduction of *rem1* hyper-recombination than

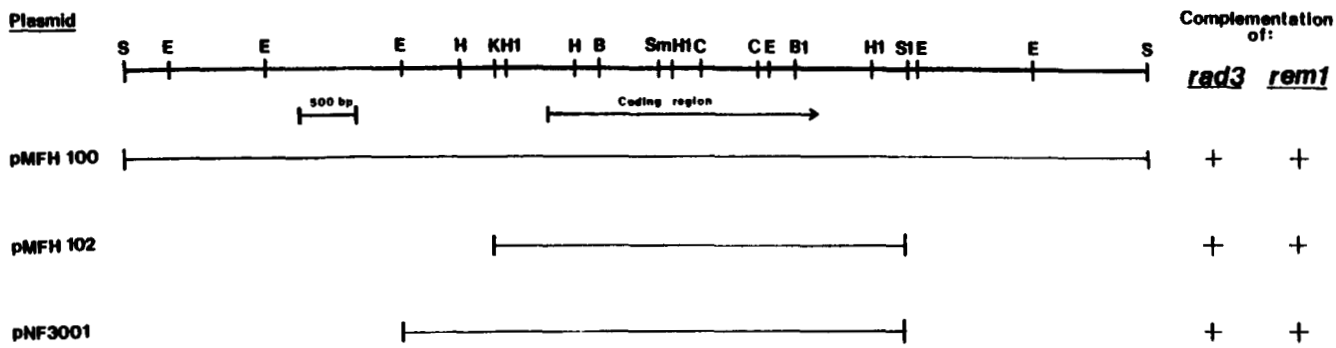

FIGURE 1.—Restriction maps and ability of various *RAD3* plasmids to complement *rad3-2* and *rem1*. pMFH100 represents a clone from a random Sau3A library of yeast DNA inserted into plasmid Yep24. This clone complemented the UV-sensitivity of a *rad3-2* strain. E is *Eco*RI; H is *Hin*dIII; K is *Kpn*I; SI is *Sal*I; B1 is *Bal*I; C is *Cla*I; HI is *Hpa*I; Sm is *Sma*I; B is *Bam*HI; S is *Sau*3AI. pMFH102 is a subclone containing the 3.9-kb *Kpn*I-*Sal*I fragment in pJ0158 (HEUTERSPREUTE *et al.* 1985). pNF3001 is a *RAD3*-containing plasmid provided by NAUMOVSKI et al. (1985). Strains containing one of the three plasmids were tested for their UV sensitivity (complementation of *rad3-2*) and mitotic recombination levels (complementation of *rem1*).

TABLE 2

The *rem1* mutations are tightly linked to *RAD3*

Genotype	Segregation pattern[a] P	T	NPD	MD[b] (cM)
$\frac{rem1\text{-}2\ +}{+\ rad4}$	59	21	2	20.2
$\frac{rem1\text{-}1\ +}{+\ rad4}$	58	14	3	21.3
$\frac{rad3\text{-}2\ +}{+\ rad4}$	89	28	4	21.4
$\frac{rem1\text{-}2\ +}{+\ rad3\text{-}2}^{c}$	26	0	0	<1.9
$\frac{rem1\text{-}1\ +}{+\ rad3\text{-}2}^{c}$	49	0	0	<1.0
$\frac{rem1\text{-}1\ +}{+\ rem1\text{-}2}^{d}$	81	0	0	<0.6

[a] P, NPD, and T refer to parental, nonparental and tetratype tetrads, respectively.

[b] Map distances were calculated using the formula MD = [(T + 6N)/(P + T + NPD)] × 100/2 (PERKINS 1949).

[c] No recombinant spores (double mutant or wild type) have been recovered out of 360 viable spores examined from both crosses. This suggests a recombination frequency <0.003.

[d] All tetrads segregated 4:0 for hyper-recombination.

did the multicopy plasmids pMFH100 and pMFH102.

Localization of the *rem1* mutations within the *RAD3* gene: The *rem1* mutations confer phenotypes differing from other known *rad3* mutations: relative UV resistance, hyper-recombination, and hyper-mutation (GOLIN and ESPOSITO 1977; MALONE and HOEKSTRA 1984; HOEKSTRA and MALONE 1987). The *rem1-2* allele is also dominant to *rad3-2*, both for UV sensitivity (Figure 2) and for mitotic recombination (Table 4). Furthermore, *rad3-2 rad52* double mutants are viable (unlike *rem1 rad52*): a diploid heterozygous for both mutations generated 63 wild type, 53 *rad3-2*, 50 *rad52-1*, and 59 *rad3-2 rad52-1* spores. Since the phenotypes of the mutant alleles are so different, we felt it was important to determine where the *rem* mutations were located within the *RAD3* gene. To this end, we performed gap-rescue experiments (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983) using various gapped derivatives of pMFH102 (our wild-type *RAD3* clone) to rescue *rem1-1* or *rem1-2*. Mitotic recombination frequencies were measured in transformed, marked diploids and are shown in Table 5 (see Figure 1 for restriction map). The rescue of *rem1-2* by a plasmid gapped with *Hpa*I (which removes the entire coding region of *RAD3*), shows a large increase in recombination frequencies at all loci, with an overall enhancement of recombination similar to that observed in *rem1-2/rem1-2* homozygotes (MALONE and HOEKSTRA 1984). This result indicates that this rescue does indeed contain the DNA sequence alteration(s) responsible for the phenotype conferred by the *rem1-2* allele. Further, two independent rescues of *rem1-2* with a *Cla*I-gapped plasmid also cause highly significant increases in mitotic recombination frequencies. These results suggest that the *rem1-2* allele may be located in or near the *Cla*I fragment. The uncertainty derives from published results indicating that gaps can be extended significant distances after transformation (*e.g.*, ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983; NAUMOVSKI and FRIEDBERG 1987). We are in the process of determining the nucleotide sequence of the *Cla*I fragment and adjacent regions of the rescued plasmids to identify any changes from wild type.

For the rescues of *rem1-1*, the situation is more complex. The *Hpa*I and *Cla-Bal* rescues show significantly elevated mitotic recombination frequencies. However, the increases are not as high as those observed with the *rem1-2* rescues. The rescues of *rem1-1* by the *Cla*I and the *Sma*I-*Cla*I gapped plasmids produced recombination frequencies not differing significantly from the strain transformed with the

TABLE 3

Effects of cloned fragments containing *RAD3* on mitotic recombination frequencies in *rem1* diploids

Strain genotype	Vector plasmid / *RAD3* plasmid	Ratio of recombination frequencies[a]						
		HIS7	*TYR1*	*LEU1*	*TRP5*	*MET13*	*CAN1*	*CYH2*
rem1-2 / *rem1-2*	YEp24 / pMFH100	50		67	34		13	49
	pJO158 / pMFH102		40	37		10	15	105
	YCp50 / pNF3001	20		15			3.6	9.4
rem1-1 / *rem1-1*	YEp24 / pMFH100			26	15		25	55
	YCp50 / pNF3001			15	6.8		7.2	9.4

[a] Values represent the ratio of geometric mean recombination frequencies for the strain transformed by the vector relative to the strain transformed by a given *RAD3*-containing plasmid. A ratio of one would indicate that the cloned fragment does not reduce mitotic recombination from the *rem1* level. The higher the ratio, the greater the reduction by the cloned fragment. pMFH100 is our original *RAD3* isolate contained in YEp24, pMFH102 is the 3.9-kb *Kpn*I-*Sal*I *RAD3* fragment subcloned in pJO158, and pNF3001 (NAUMOVSKI *et al.* 1985) is a 4.5-kb *Eco*RI-*Sal*I *RAD3* fragment in YCp50. For YEp24 and YCp50-based plasmids, 12 cultures were grown. For the pJO158-based plasmids, 15 cultures were grown. All experiments were performed on uracil omission (YEp24 and YCp50) or tryptophan omission (pJO158) medium to ensure maintenance of the plasmid.

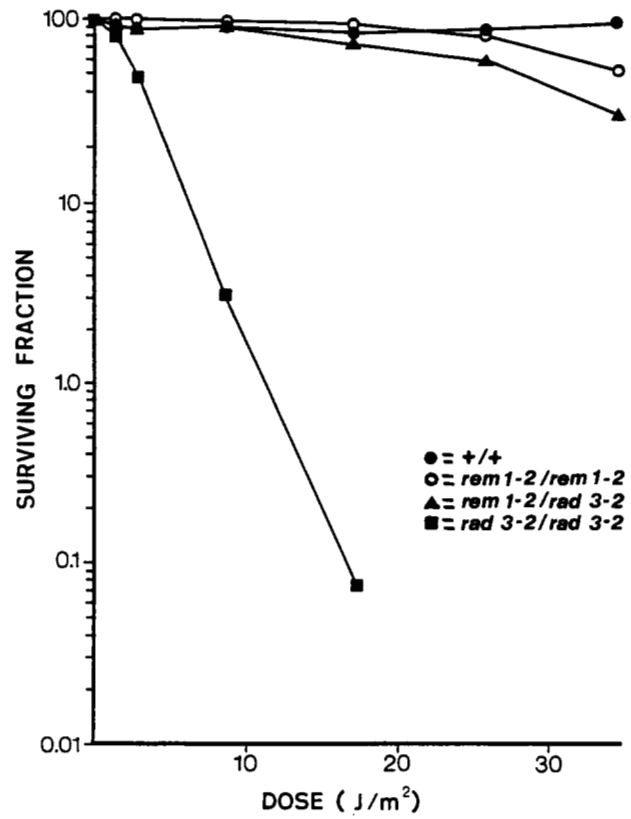

FIGURE 2.—The *rem1-2* mutation is dominant to *rad3-2* for UV sensitivity. UV survival curves were performed as described in MATERIALS AND METHODS. Diploid strains are the same as those described in Table 4.

TABLE 4

Comparison of mitotic recombination in diploid strains containing *rem1-2* and *rad3-2*

Diploid genotype	Relative recombination frequencies[a]					
	Intragenic					Intergenic
	URA3	*HIS7*	*TYR1*	*LEU1*	*LYS2*	*CYH2*
+ / +	1.0	1.0	1.0	1.0	1.0	1.0
rad3-2 / *rad3-2*	1.1	1.6	1.7	—	0.43	2.4
rem1-2 / *rad3-2*	6.3	11	25	17	12	9.0
rem1-2 / *rem1-2*	5.4	13	36	24	16	8.1

[a] Values are normalized to the wild-type recombination frequencies presented in Table 6. The *rem1-2* and *rad3-2* strains used to construct the diploids were sibling segregants from the mapping crosses described in Table 2.

wild-type *RAD3* plasmid alone. These results suggest that neither the *Sma*I-*Cla*I nor the *Cla*I fragment contain *rem1-1*. The mutation may therefore lie within the *Cla*I-*Bal*I fragment or 3′ to the *Bal*I site, since the *Cla*I-*Bal*I rescue showed the *rem1* phenotype. These results also indicate that overexpression of the wild-type *RAD3* gene can elevate mitotic recombination (compare the parent strain with and without the *RAD3* plasmid in Table 5).

TABLE 5

Relative mitotic recombination frequencies in wild-type strains bearing various rescued plasmids

Allele rescued	Gap	Relative recombination frequencies							
		TYR1	*LYS2*	*TRP5*	*LEU1*	*MET13*	*CAN1*	*CYH2*	"X"[a]
rem1-2	*Hpa*I-*Hpa*I[b]	325[c]	56.3*	29.6*	23.4*	30.2*	9.3*	10.8*	26.6
rem1-2	*Cla*I-*Cla*I	38.3*	33.8*	18.1*	15.4*	39.7*	8.6	8.4	23.2
rem1-2	*Cla*I-*Cla*I	14.0*	20.6	10.9*	19.4	13.7*	5.0*	8.2*	13.1
rem1-1	*Hpa*I-*Hpa*I[b]	6.4*	11.9*	10.2*	6.4*	6.6*	2.3	6.6*	7.2
rem1-1	*Cla*I-*Bal*I	11.4*	21.8*	8.6*	6.9*	7.6*	3.5*	5.1	9.3
rem1-1	*Cla*I-*Cla*I	4.7	2.0	8.4*	5.3*	5.1*	3.5	3.7	4.7
rem1-1	*Sma*I-*Cla*I	5.5	1.7	1.9	2.8	5.1*		3.3	3.4
	None[d]	4.0#	6.5#	2.0#	1.5	2.2	2.2#	1.8	2.9
	None (parent)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
		(1.04)	(1.44)	(12.6)	(20.3)	(12.4)	(324)	(289)	

All plasmids were present in the *RAD3* diploid whose recombination values are shown in the bottom row. Geometric means were calculated from five independent cultures for each strain. The numbers in parentheses under the parent strain are the actual mean frequencies × 10^6. The other values have been normalized to the parent strain frequencies.

[a] "X" is an arbitrary measure of the effect of the plasmid on mitotic recombination. It represents the average increase over the parent for all loci measured.

[b] The *Hpa*I-*Hpa*I rescues span the complete coding region of *RAD3*. See Figure 1 for the restriction map of *RAD3*.

[c] Mean value is very high because of "jackpot" events in two cultures. Not used to calculate "X" for this rescue.

[d] Intact *RAD3* plasmid pMFH102CBU.

* Represents a mean recombination frequency differing significantly ($P < 0.05$) as determined by Student's *t*-test from the mean measured in the pMFH102CBU-bearing strain.

Represents a mean recombination frequency in the pMFH102CBU-bearing strain that is significantly different ($P < 0.05$) from the parent wild-type strain with no plasmid.

Increased gene conversion in *rem1* cells requires excision-repair functions: The lethality of *rem1* in combination with the dsb repair functions *RAD50* and *RAD52* suggests dsb's may occur in *rem1* strains. However, since *rem1* strains also display increased mutation rates (HOEKSTRA and MALONE 1987), the initial lesion seemed unlikely to be a dsb, since repair of dsb's is not mutagenic (HAYNES and KUNZ 1981). Instead, we proposed that the initial lesion occurring in *rem1* strains was acted upon by an unknown function, and that action ultimately led to a dsb (HOEKSTRA and MALONE 1987).

Genes in the excision-repair system seemed to us to be good candidates for the unknown function. Not only can they detect and act on pyrimidine dimers and other bulky lesions such as psoralen adducts (JACHYMCZYK *et al.* 1981; MAGANA-SCHWENCKE *et al.* 1982; MILLER, PRAKASH and PRAKASH 1982) but they are capable of recognizing small adducts like *N*-6-methyladenine (HOEKSTRA and MALONE 1986). We therefore constructed double mutants with *rem1* and the excision-defective mutations *rad1-2* and *rad4*. The double mutants, *rem1 rad1-2* and *rem1 rad4*, were completely viable (data not shown). However, *rad1* and *rad4* unexpectedly reduced the level of gene conversion in *rem1* strains to essentially the level seen in the excision-repair mutants alone (Table 6). This implies that the excision-repair functions are required for this part of the hyper-rec phenotype of *rem1*. The *rem1* level of intergenic crossing over, as measured by drug resistance at *CAN1* and *CYH2*, was not reduced by the excision-repair defects.

To demonstrate that the reduction in gene conversion was not due to reversion of *rem1* in the strains used, a number of recombinant colonies from these experiments were sporulated and tetrads dissected. In all cases (10 of 10 asci generating four live spores) the segregants demonstrated both the *rem1* and *rad1* (or *rad4*) phenotypes (data not shown). A second experiment confirmed that the selected prototrophs were actually convertants and not crossover events. Ten Ura$^+$ and ten Leu$^+$ colonies from each mutant class presented in Table 6 were sporulated and dissected. In all cases, the progeny demonstrated that greater than 95% of the mitotic events for the *ura3* or *leu1* heteroalleles in each strain class must have been gene conversions since reciprocal double mutants were not observed (data not shown). Therefore, the hyper-gene conversion observed in strains containing *rem1* requires at least the *RAD1* and *RAD4* functions.

Elevated drug-resistance frequencies observed in *rem1 rad1* and *rem1 rad4* diploids are not due to chromosome loss: It was surprising that the excision-repair mutations reduced *rem1* gene conversion but not the frequency of drug resistance (presumed to be due to crossing over). Current molecular models of mitotic and meiotic recombination propose that gene conversion and crossing over are associated events (MESELSON and RADDING 1975; ESPOSITO and WAGSTAFF 1981; SZOSTAK *et al.* 1983). To verify that the drug-resistant colonies used as a measure of crossing over did not represent chromosome loss events, we constructed strains designed to simultane-

TABLE 6

Spontaneous mitotic recombination in excision-repair deficient *rem1*-containing strains

Genotype	No. cultures[a]	Relative recombination frequencies								
		Intragenic							Intergenic	
		LYS2	*TYR1*	*HIS7*	*URA3*	*MET13*	*TRP5*	*LEU1*	*CAN1*	*CYH2*
$\frac{+}{+}$	19,23	1.0 (0.4)	1.0 (0.3)	1.0 (0.36)	1.0 (0.51)	1.0 (4.2)	1.0 (3.1)	1.0 (3.2)	1.0 (22)	1.0 (41)
$\frac{rem1\text{-}2}{rem1\text{-}2}$	9,19	16	36	13	19	8.8	8.9	24	7.4	8.1
$\frac{rad1\text{-}2}{rad1\text{-}2}$	3,9		1.7	1.1	0.9			1.8	5.2	3.2
$\frac{rad4}{rad4}$	6,12	0.55	0.36	0.55	0.83	1.5	1.5	1.0	7.5	7.4
$\frac{rem1\ rad1}{rem1\ rad1}$	6,16		1.2	1.6	0.62	0.91			10	11
$\frac{rem1\ rad4}{rem1\ rad4}$	6,16	0.65	1.6	1.5	1.7	1.3	1.9	2.3	11	3.3

Recombination levels are geometric mean frequencies normalized relative to wild-type levels. The wild-type recombination frequencies ($\times 10^5$) are given in brackets below the first row.

[a] The first value indicates the number of cultures examined for intragenic recombination (gene conversion). The second number indicates the number of cultures examined for intergenic recombination (crossing over).

TABLE 7

Chromosome loss in strains bearing *rem1* or *rad* mutations

Diploid genotype	No. cultures	Chromosome *V*			Chromosome *VII*		
		Total *can1*^{R} frequency ($\times 10^6$)	Frequency due to loss[a] ($\times 10^6$)	Percent loss[b]	Total *cyh2*^{R} frequency ($\times 10^6$)	Frequency due to loss[a] ($\times 10^6$)	Percent loss[b]
$\frac{+}{+}$	12	300	2.8	0.93	310	2.1	0.66
$\frac{rad1\text{-}2}{rad1\text{-}2}$	6	1000	29	2.8	790	1.4	0.17
$\frac{rad4}{rad4}$	8	1500	17	1.1	1200	18	1.5
$\frac{rem1\text{-}2}{rem1\text{-}2}$	8	2700	23	0.85	5400	29	0.55
$\frac{rem1\ rad1}{rem1\ rad1}$	8	1600	7	1.1	1200	6.5	0.54
$\frac{rem1\ rad4}{rem1\ rad4}$	8	2000	15	0.74	2200	4.7	0.21

The level of chromosome loss contributing to the drug-resistant population was determined as described in the text.

[a] Frequency of chromosome loss resulting in drug resistance.

[b] Relative amount of chromosome loss occurring in a drug-resistant population.

ously measure crossing over and chromosome loss (see MATERIALS AND METHODS). Table 7 gives the level of chromosome loss in wild-type, *rem1*, *rad1-2*, *rad4* and double mutant strains. While chromosome loss relative to wild type is elevated approximately tenfold in all mutant strains, the *rem1-2 rad1-2* and *rem1-2 rad4* double mutants show *no more* chromosome loss than the single mutants. The level of chromosome loss in wild-type strains is similar to values reported by others for chromosomes *V* and *VII* (MALONE, GOLIN and ESPOSITO 1980; ESPOSITO *et al.* 1982; HARTWELL *et al.* 1982). Therefore, the level of drug-resistant colonies used to measure intergenic recombination in these strains is an accurate indicator of the level of crossing over. We conclude that mutations in excision-repair functions specifically reduce *rem1*-

TABLE 8

Excision-repair mutations rescue the inviability of *rem1 rad50* and *rem1 rad52* double mutants

Diploid genotype	Segregant genotype							
	RADX[a] *RADY*[b] *REM1*	*RADX RADY rem1*	*RADX radY REM1*	*radx RADY REM1*	*RADX radY rem1*	*radX radY REM1*	*radX RADY rem1*	*radX radY rem1*
$\frac{rem1}{+}\ \frac{rad1}{+}\ \frac{+}{rad52}$	24	31	26	22	0	30	26	26
$\frac{rem1}{+}\ \frac{rad1}{+}\ \frac{+}{rad50}$	31	36	24	18	0	32	35	29
$\frac{rem1}{+}\ \frac{rad4}{+}\ \frac{+}{rad52}$	57	6	40	4	0	10	50	45
$\frac{rem1}{+}\ \frac{rad4}{+}\ \frac{+}{rad50}$	47	6	38	8	0	8	40	41

The mutations used in this experiment were *rem1-2, rad1-2, rad50-1,* and *rad52-1*. Triply heterozygous diploids were constructed, sporulated, dissected, and viable spores tested for the presence of *rem1* and/or *rad* mutations. The eight possible segregant genotypes are presented.

[a] "X" refers to the excision-repair mutation in the diploid.

[b] "Y" refers to the recombination-repair mutation in the diploid.

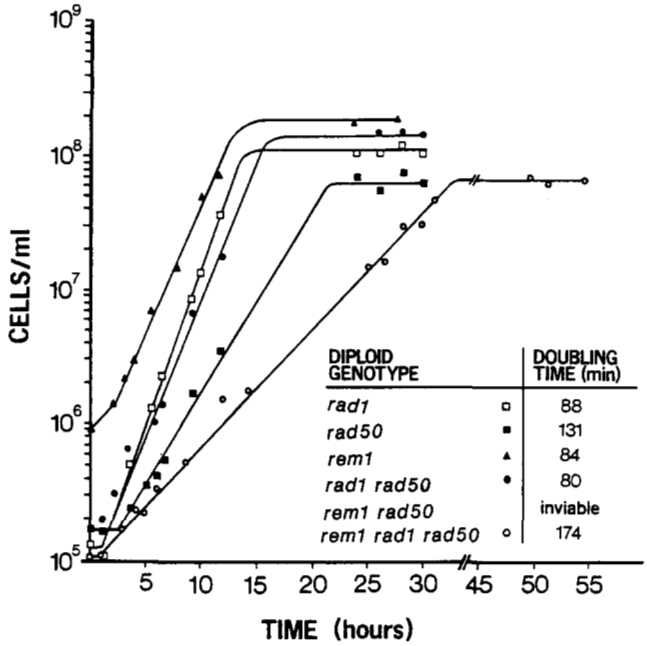

FIGURE 3.—Growth curves and doubling times in various *rem1* and *rad*-containing strains. Cell counts were made from duplicate hemocytometer readings at various time points. Doubling times for related wild type strains (not shown) averaged 80 min.

elevated gene conversion but do not reduce *rem1*-elevated crossing over.

Excision-repair mutations prevent the lethality of *rem1 rad50* and *rem1 rad52*: We have previously proposed that *rem1* strains contain a recombinogenic lesion requiring dsb repair functions for resolution (MALONE and HOEKSTRA 1984). As described above, *rem1* hyper-gene conversion requires excision-repair functions. We therefore asked whether these two sets of interactions were related. In other words, do *RAD1* and *RAD4* act on the initial *rem1* lesion in a fashion which both stimulates gene conversion and causes a requirement for recombination-repair? Triple mutants with *rem1-2* in combination with *rad1-2* (or *rad4*) and *rad50-1* (or *rad52-1*) are indeed viable (Table 8). We conclude that mutations in at least two excision-repair genes prevent the occurrence of the lesion requiring the recombination-repair genes. Although viable, the triple mutants grow slowly (Figure 3). This suggests that the triple mutants have difficulty in "processing" the *rem1* lesion. It is interesting that a defect in excision repair restores the growth rate of *rad50* strains to that of wild type (Figure 3); this may indicate an interaction between the two pathways even in wild-type cells.

Analysis of recombination in triple mutants: The results described above indicate that *RAD1* and *RAD4* are necessary for the increased gene conversion, but not the high levels of crossing over, observed in *rem1* strains. Mutations in *rad1* or *rad4* also obviate the need for recombination-repair. Taken together, these observations indicate that the elevated crossing over observed in *rem1* strains might be independent of the recombination-repair pathway. To test this possibility, we examined mitotic recombination in the triple mutant *rem1-2 rad1-2 rad52-1*. As shown in Table 9, the triple mutants are reduced for gene conversion but *not* for the frequency of drug-resistant colonies. [A *rem1-2 rad4 rad52-1* strain gave similar results (data not shown).] Because *rad52-1* increases chromosome loss (MORTIMER, CONTOPOULOU and SCHILD 1981) we asked what effect it had in the *rem1 rad1* background (Table 10). Although there is indeed a great deal of chromosome loss occurring in triple mutants, the residual drug-resistance frequency attributable to crossing over is approximately that of wild-type cells. Since no elevation of crossing over was seen, we conclude that recombination-repair (or at least

TABLE 9

Spontaneous mitotic recombination in a triple mutant

Diploid genotype	No. cultures	Relative recombination frequencies							
		Intragenic						Intergenic	
		URA3	*HIS7*	*TYR1*	*LYS2*	*LEU1*	*TRP5*	*CAN1*	*CYH2*
rad52/*rad52*	6[a]	0.13	0.13	0.03	0.11	0.009	0.018	0.45[b]	0.18
rad1 rad52/*rad1 rad52*	6		0.25	0.14	0.40			68	9.8
rem1 rad1 rad52/*rem1 rad1 rad52*	6	0.051	0.15	0.042	0.20	0.034[c]	0.044[c]	135	13

The alleles used are *rem1-2*, *rad1-2*, and *rad52-1*. Values are normalized to the wild type values given in Table 6.
[a] Data from MALONE and ESPOSITO (1980).
[b] Data from MALONE and ESPOSITO (1981).
[c] Data from 10 cultures of a different triple mutant strain (the strain used in the chromosome loss experiment in Table 10).

TABLE 10

Mitotic crossing over and chromosome loss frequencies in wild-type and *rem1 rad1 rad52* triple mutant strains

A. Proportion of chromosome loss among individual drug-resistant papillae

Genotype	No. *can*^{r} tested	No. *his1 can*^{r}	% loss	No. *cyh*^{r} tested	No. *ade6 cyh*^{r}	% loss
Wild type	147	3	2.0	144	7	4.9
Triple mutant	210	197	93.8	204	109	53.4

B. Mitotic crossover frequency corrected for chromosome loss

Genotype	Marker	Observed frequency resistance	Proportion due to loss	Corrected exchange frequency
Wild type	*CAN1*	3.22×10^{-4}	6.44×10^{-5}	3.16×10^{-4}
Triple mutant	*CAN1*	1.10×10^{-2}	1.03×10^{-2}	6.82×10^{-4}
Wild type	*CYH2*	4.51×10^{-4}	2.21×10^{-5}	4.29×10^{-4}
Triple mutant	*CYH2*	2.77×10^{-4}	1.48×10^{-4}	1.29×10^{-4}

Individual drug-resistant papillae from independent colonies were picked and tested for expression of recessive markers linked on the opposite side of the centromere as described in the text. The resulting percentages of drug resistance due to loss were used to adjust the drug-resistance frequencies [calculated as the geometric means of 9 (for wild type) or 10 (for triple mutant) individual liquid cultures] to obtain a corrected exchange frequency.

RAD52) is required for at least some *rem1* hyper-crossing over. Since the cells are viable, we also conclude that this crossing over is unlikely to be stimulated by dsb's (see DISCUSSION).

Physical evidence for dsb's in *rem1* cells: Several of the results above suggest that the lesions caused by *rem1* can be converted to dsb's by the action of the excision-repair system. To test this hypothesis we examined *rem1* DNA on neutral sucrose gradients. Consistent with the genetic evidence, the profile of *rem1* DNA on neutral sucrose gradients is shifted to smaller sizes than DNA of an isogenic wild type (Figures 4 and 5). All experiments were done with *rem1* and wild-type cells differentially labeled, mixed together, and then lysed and analyzed on the same gradients to avoid artifacts. The curve in Figure 4 is a representative neutral sucrose gradient of DNA prepared from strains which have had the label chased for a generation after an overnight pulse. The number average molecular weight (M_n) for the furthest sedimenting chromosomal peak in *rem1-2* is 2.18×10^8 daltons while in the *REM1* diploid strain it is 2.65×10^8 daltons (calculated from an average of three gradients). The calculated M_n for wild type is reasonably similar to the value of $3.0 \pm 0.3 \times 10^8$ reported by RESNICK and MARTIN (1976). Since the strains used in the experiments shown in Figure 4 contained mitochondrial DNA, we could not examine the sizes of newly synthesized DNA in wild-type and *rem1* strains. Therefore we isolated "petite" derivatives by growth in the presence of ethidium bromide (SLONIMSKI, PERRODIN and CROFT 1968). Newly replicated DNA from these strains was examined by growing overnight in the presence of label with no chase followed by sedimentation on neutral sucrose gradients. Figure 5 demonstrates a normalized plot for eight gradient runs. To generate this figure we have taken the ratio of *REM1*:*rem1* per gradient

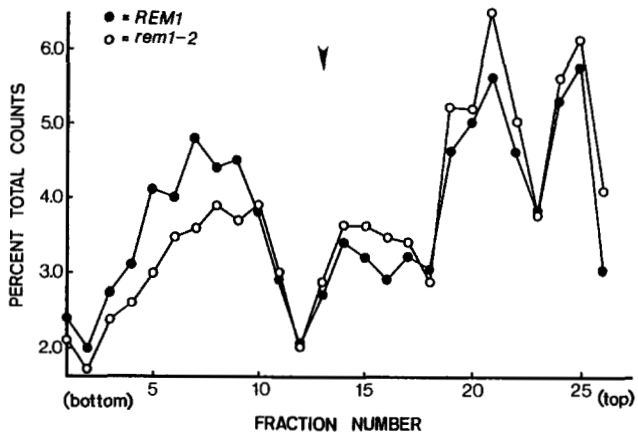

FIGURE 4.—Sucrose gradient analysis of *rem1* cells. *REM1* and *rem1-2* cells were grown overnight in synthetic medium containing [^{3}H]- or [^{14}C]adenine as described in MATERIALS AND METHODS. Sucrose gradients (5–20%) were formed and run as described in MATERIALS AND METHODS. Phage T4 DNA was used as a size standard and its sedimentation position is indicated (*arrowhead*). Calculated number average M_n were 2.65×10^8 daltons for *REM1* and 2.18×10^8 daltons for *rem1*.

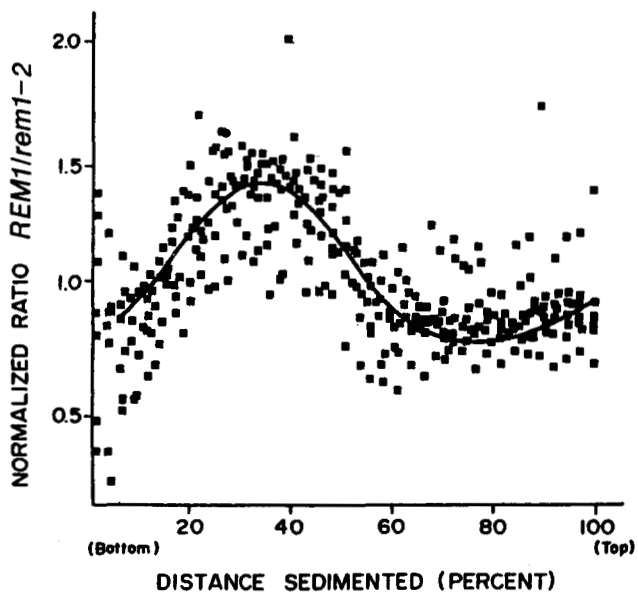

FIGURE 5.—Neutral sucrose gradient analysis of *rem1*. Petite *REM1* and *rem1-2* cells lacking mitochondria were labeled and run on 5–20% neutral sucrose gradients as described in MATERIALS AND METHODS. The normalized ratio of *REM1*/*rem1-2* for eight separate gradients is plotted as a function of sedimentation. To generate this figure we have taken the ratio of *REM1*:*rem1* per gradient fraction and normalized to the ratio of total counts per gradient. This sets a normalized value of unity if the relative amount of DNA at a point in the gradient is equal in both strains. Regions of the curve greater than 1.0 indicate more *REM1* DNA is present compared to *rem1*. Values less than 1.0 indicate the amount of DNA from *rem1* is greater than *REM1*. In these experiments the *rem1* cells were labeled with ^{14}C and the wild-type cells with ^{3}H. Experiments with the labels reversed gave similar results.

fraction and normalized to the ratio for the total counts per gradient. Therefore, values greater than 1.0 indicate more *REM1* DNA is present than *rem1*. Values less than 1.0 indicate the amount of DNA from *rem1* at that point is greater than wild type. Interpolating our values for *rem1-2* and *REM1* with published dose curves (RESNICK and MARTIN 1976), it appears as if the change in M_n is similar to an X-ray dose of approximately 5 krad. In a wild-type cell, a dose of 5 krad reduces viability a few percent at most, while in *rad52* cells, viability is reduced by two to three orders of magnitude (GAME and MORTIMER 1974; RESNICK and MARTIN 1976). This is consistent with the genetic observation of double mutant inviability. This dose also corresponds to approximately one to two strand breaks/cell (RESNICK and MARTIN 1976).

DISCUSSION

In this report we have demonstrated that the hyper-recombination and hyper-mutation causing mutations *rem1-1* and *rem1-2* are alleles of the essential gene *RAD3*. Both genetic mapping and complementation with cloned genes indicate that the *rem1* mutations are alleles of *RAD3*, and we therefore propose that *rem1-1* be known as *rad3-101* and *rem1-2* as *rad3-102*.

Why was the identity of the *rem1* mutations not discovered earlier? Because the *rem1* phenotypes are very different from those of the UV-sensitive *RAD3* mutations, there was no reason to suppose that *rem1* might be an allele of a UV repair gene. The *RAD3* gene is an essential mitotic function (HIGGINS *et al.* 1983; NAUMOVSKI and FRIEDBERG 1983) involved in the incision step of excision-repair (REYNOLDS and FRIEDBERG 1981; WILCOX and PRAKASH 1981). These two groups originally cloned and sequenced *RAD3* (NAUMOVSKI and FRIEDBERG 1982, 1983; HIGGINS *et al.* 1983; NAUMOVSKI *et al.* 1985; REYNOLDS *et al.* 1985). Recent data indicate that at least one function of the Rad3 protein is a DNA-dependent ATPase (SUNG *et al.* 1987). The many phenotypes exhibited by mutant alleles of *RAD3* also suggest that it may encode a multifunctional protein; however, to date, no one region has been absolutely defined mutationally as being responsible for the UV repair or essential functions (NAUMOVSKI and FRIEDBERG 1986, 1987). Localizing the *rem1* alleles, which differ phenotypically from other *rad3* mutant alleles, should help to elucidate the structure-function relationships of this important protein. The rescue experiments suggest that at least *rem1-2* is located in or near the *Cla*I fragment; this region is of particular interest because it contains the portion of sequence identified as resembling sequences encoding DNA-binding domains of other proteins (NAUMOVSKI *et al.* 1985; REYNOLDS *et al.* 1985; NAUMOVSKI and FRIEDBERG 1986).

There are several precedents for "DNA repair genes" coding for products involved in various aspects of DNA metabolism. One example is that of the excision repair gene *uvrD* of *E. coli* (CARON,

KUSHNER and GROSSMAN 1985; HUSAIN *et al.* 1985) which is now known to encode the ATP-dependent DNA helicase II (KUMURA and SEKIGUCHI 1984). Mutations in *uvrD* have been variously isolated as UV-sensitive, as spontaneous mutators, or as hyper- or hypo-rec mutants (OGAWA, SHIMADA and TOMIZAWA 1968; SMIRNOV and SKAVRONSKAYA 1971; SIEGEL 1973; HORII and CLARK 1973; KONRAD 1977). This array of phenotypes is reminiscent of those associated with the various *rad3* alleles, and suggests one possible function for the wild-type *RAD3* gene product, particularly in light of the discovery of its ATPase activity (SUNG *et al.* 1987).

Since *rem1 rad52* and *rem1 rad50* double mutants are inviable (MALONE and HOEKSTRA 1984), we proposed that recombinogenic lesions occur in *rem1* strains that require resolution by the recombination-repair epistasis group. The simplest explanation for the lesion would be a dsb. However, since the recombination-repair system does not appear to create mutations while repairing dsb's (HAYNES and KUNZ 1981), this hypothesis did not easily explain the increased mutation frequency of *rem1* strains. We then proposed that the lesions require processing to form dsb's. We found that triple mutants with blocks in excision- and strand-break-repair (*e.g., rem1 rad1 rad52*) are alive. This suggests that the excision-repair functions act on the initial lesion to ultimately produce dsb's in *rem1* strains.

The viability of triple mutants has allowed us to examine the levels of recombination in these strains. The *rad52-1* mutation confers a mitotic Rec$^-$ phenotype for both gene conversion and, to a lesser extent, crossing over between homologous chromosomes (MALONE and ESPOSITO 1980; PRAKASH *et al.* 1980; HOEKSTRA, NAUGHTON and MALONE 1986). Thus, triple mutants such as *rem1 rad1 rad52* should demonstrate *rad52* levels of conversion and crossing over if *rem1* hyper-recombination were entirely dependent on *RAD52*. As shown in Table 10, triple mutants demonstrate greatly reduced levels of gene conversion, but wild type levels of crossing over, intermediate between *rem1* and *rad52-1* levels. These crossovers must by definition be occurring by some pathway other than the *RAD52* recombination-repair mode. Observations by several laboratories suggest the existence of such a pathway for recombination between sister chromatids in the ribosomal DNA, for intrachromosomal events between duplicated genes, and for integration of a circular plasmid into its homologous chromosomal site (JACKSON and FINK 1981; ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; PRAKASH and TAILLON-MILLER 1981; ZAMB and PETES 1981). Consistent with these results, HABER and HEARN (1985) proposed that in *rad52-1* strains gene conversion associated with crossing over occurs by a pathway distinct from that responsible for conversion alone.

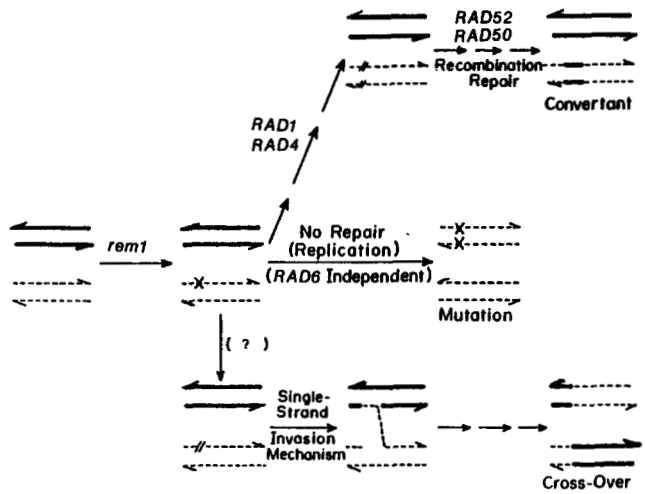

FIGURE 6.—Model for interactions between *rem1* and various repair mutations leading to the production of gene conversions, crossovers, and mutations.

Figure 6 is an interpretation of the interactions described in this report. We propose that *rem1* strains contain DNA lesions, indicated as "X," that can stimulate mutation, and if acted upon by excision-repair, recombination. We propose three alternative fates for the lesion in Figure 6: (1) The lesion is recognized and acted upon by functions including *RAD1* and *RAD4*. (The actual number and nature of the steps are not known and have been designated by three arrows.) During this process, a dsb may form that requires *RAD50* and *RAD52* for resolution, generating "*rem1* hyper-recombination." In the absence of *RAD1* or *RAD4*, recombinogenic dsb's are not formed and the recombination-repair system is not needed for survival. (2) An alternative fate of "X" is to become fixed as a mutation, presumably by DNA replication. [Neither the excision-repair functions nor *RAD6* function is necessary for "*rem1*-hypermutation" (HOEKSTRA and MALONE 1987).] (3) Finally, "X" may initiate a recombinational process generating the crossovers seen in *rem1*-excision-defective double mutants and in the triple mutants containing *rad52-1*. Although the *RAD52* gene is clearly not required for this recombination, when it is present, it may contribute to recombinants formed in this way. We argue that this crossing over cannot involve a dsb because it occurs in the absence of *RAD52*; the third pathway shown in Figure 6 suggests that it might involve a single strand exchange mechanism (*e.g.,* MESELSON and RADDING 1975).

What is the identity of the *rem1* DNA lesion? Given the allelism of *rem1* with *RAD3*, the fact that *RAD3* is essential, and the behavior of the multiple mutants, it is reasonable to suppose that it is an aberrant product of DNA replication, perhaps a base mismatch. [Mismatch repair has recently been shown to occur in mitosis in wild-type yeast (BISHOP and KOLODNER 1986; BISHOP *et al.* 1987).] The hyper-mutational phenotype of *rem1* mutants would be easily

explained by such a hypothesis. To account for the hyper-recombinational phenotype of *rem1* and the multiple mutant results, the model in Figure 6 needs only the assumption that excision repair functions in yeast can recognize some subset of DNA replication errors. Since they can recognize adenine methylation (HOEKSTRA and MALONE 1986), a relatively subtle change, it is perhaps not an unreasonable assumption. DICAPRIO and HASTINGS (1976) reported that *rad1* and *rad4* did not affect the frequency of postmeiotic segregation. However, it is not clear that mismatches created during meiotic recombination and mismatches created during mitotic DNA replication would be repaired by the same systems. It has not been reported whether *RAD1* and *RAD4* are even expressed in meiotic cells.

The model predicts that the *rem1* lesion is a DNA replication error, suggesting that the role of the wild-type *RAD3* gene product may be in maintenance of the fidelity of DNA replication. The properties of the *rem1* alleles may provide us with a unique opportunity to study the role of Rad3 protein *in vivo* and *in vitro*.

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